STUDIES ON THE RELATION OF THE LIVING CELLS TO THE TRANSPIRATION AND SAP-FLOW IN CYPERUS. II

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(WITH TWO FIGURES)

Experiments with poisons

In order to kill certain portions of the stems of *Cyperus*, I have used chloroform and ether in the manner described by ROSHARDT, and have always found that the leaves soon droop, behaving exactly like those placed in an atmosphere of these substances. When the fluid is poured into a tube incasing the stem or placed on cotton in the tube, the leaves soon droop. This indicates in my opinion that these substances are soon absorbed and carried to the leaves. The leaves soon fade and wither after treatment with ether or chloroform. I have also made several experiments with xylol in the same way, and found that the results are similar to those obtained by using steam. In one experiment xylol containing eosin was poured into an incasing tube inclosing 15 cm. of a stem 43 cm. high. That the xylol was absorbed by the stem was indicated by the color; at the end of 30 min. the xylol was drawn off and the joints were secured. A plant had been chosen which had a well-developed branch from the base of one of the crown leaves. The involucre remained fresh and turgid for 10 days and then dried, while the branch lived 5 days longer; the part treated with xylol collapsed and the leaves became yellow and dried in about 15 days. The whole behavior of the plant was like that of one which had had a portion of its stem steamed, except that leaves on such steamed plants do not always turn yellow. Other experiments with xylol gave similar results.

I have tried several other poisons in solution, killing 5–10 cm. of the stems by pouring the liquid into incasing tubes. In many cases I have chosen plants with branches developing from the crown. I have used 95 per cent alcohol, 1 per cent chromic acid, saturated

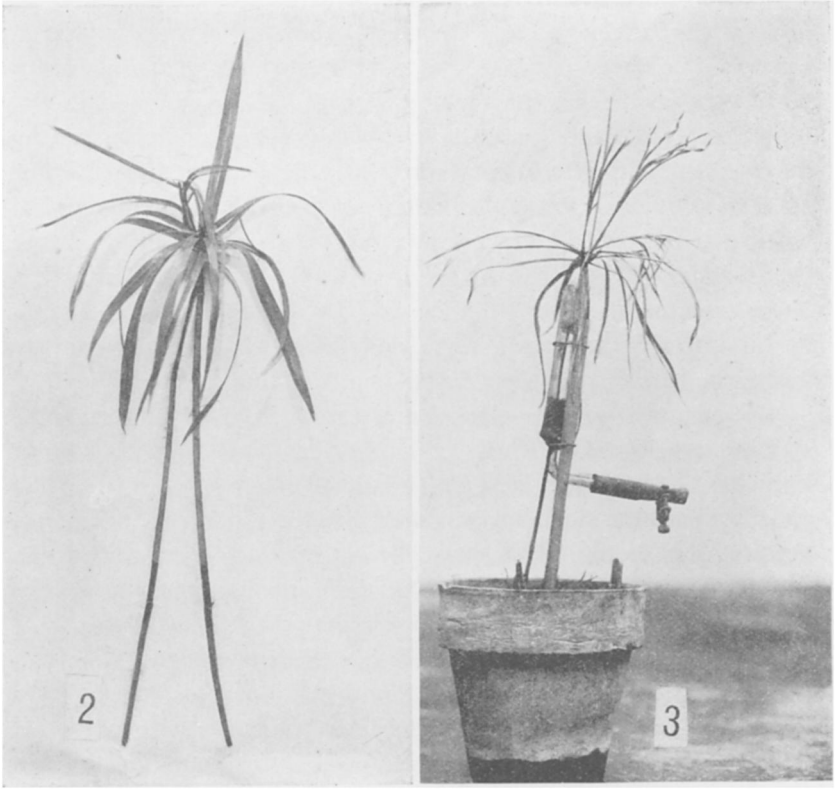
aqueous solutions of $HgCl_2$, $CuSO_4$, and picric acid, Zenker's fixing fluid, KOH, and 40 per cent formalin for 0.5–72 hours.

In one case 10 cm. of a 40 cm. stem were surrounded for 48 hours with picric solution; the involucral leaves drooped in 3 days, but were perfectly turgid and did not become discolored and dry for 26 days, and the branches, which were 10 cm., 11 cm., and 12 cm. long, remained fresh for 44 days. The incasing tube was then removed and the plant was apparently in good condition. The stem was obviously dead, the immersed region being discolored, but there was no contraction of the protoplasts observable. Other experiments with picric acid are described in table X below.

In experiments with chromic acid, $HgCl_2$, Zenker's fluid, formalin, and KOH, the plants very soon faded, although not always losing the turgidity of their leaves immediately after treatment.

By using 95 per cent alcohol and $CuSO_4$ I have obtained some striking results. On April 12 a plant 35 cm. high, with four branches on the crown, was chosen and the stem incased in a glass tube; 95 per cent alcohol was poured into the tube so as to immerse the stem for 9 cm. The lower portion of the treated region was slightly mechanically injured, so that the alcohol could be readily absorbed; that it was absorbed was shown by a lowering of the fluid in the tube. At the end of 48 hours the leaves of the crown had shown signs of drooping and the liquid was removed; after 7 days the crown leaves and two branches were partially withered. Six of the crown leaves faded and yellowed in 10 days, one branch showed one leaf, and one branch showed 2 leaves alive for 6 weeks. The remaining crown leaves continued green, with but portions yellow, and the remaining 2 branches kept on growing, and at this writing (July 18), after 76 days, were 6 and 8 cm. long respectively, having grown about 5 cm. each. Furthermore, 10 days after treatment, 7 new branches started, all of which were growing (July 18). The incasing tube was removed from the stem, and it was examined to be sure a section was dead. As to this there can be no question, as is shown by the photograph (fig. 2). As a result of this examination, it was found that 4 cm. of the stem where injured were entirely brown and the parenchyma disorganized, which appears black in the figure, but apparently no substance had been carried into the

lumina of the vessels. No streaks could be observed above the dead part, as is the case in stems on which steam is used. Apparently



FIGS. 2, 3.—Fig. 2, photograph of a plant which has had a portion of the stem killed with 95 per cent alcohol; stem split lengthwise in order to show the appearance of the dead portion; dead cells appear black in photograph; parenchyma cells almost entirely disintegrated; this figure also shows the healthy crown of leaves and several new branches which have developed since the treatment and which were still growing at the time when the stem was cut; fig. 3, photograph of a plant 63 days after 10 cm. were killed with $CuSO_4$; incasing tube into which the poison was poured still in place; darkened, shriveled stem seen through glass tube surrounded with a plug of cotton; crown shows several leaves still in good condition and the growing branch in center still turgid.

the plant would have remained alive and the stem have continued to conduct sap as long as the injured portion was protected. The dead portion fell to pieces when exposed and touched. From this

experiment, therefore, it seems possible to kill a certain region of the stem without injuring the leaves, and to show that the dead portion is capable of water conduction so long as actual breaking up can be prevented.

The objection may be raised that the killed region in the above experiment was too short to show conclusively that living cells are not necessary for sap-flow. I have succeeded in killing 10 cm. of the stem by another poison without injury to all of the parts above, and thus obtaining results quite as striking. A stem 23 cm. long, with a healthy crown of 11 involucral rays with 2 branches each 8 cm. long and 2 others each 10 cm. long, was surrounded for 4 cm. with a saturated aqueous solution of $CuSO_4$ for 36 hours on May 7. The $CuSO_4$ was absorbed and the stem discolored for 20 cm., or up to the base of the crown. The liquid was removed and a plug of cotton put loosely about the stem in the tube inclosing the upper portion of the 10 cm. which was discolored. On May 20, or after 13 days, the plant was in perfect condition except one branch, some of whose leaves were drying, and the leaf tips of the crown, which showed signs of yellowing. This branch and one of the others finally dried and died, and 3 leaves of the crown died, but the remaining 8 leaves of the crown and the branch have remained perfectly fresh, green, and turgid up to the present writing (July 18); the other branch has grown several centimeters. The stem is partially collapsed, as indicated by its smaller size, and has colonies of mold (*Penicillium*) on it, owing to the fact that the incasing tube was not closely sealed. The stem is dark brown, almost black, and is certainly dead. This experiment shows, therefore, that it is possible for 10 cm. of a dead stem to conduct water for 8 leaves and 2 branches for an indefinite period of time. A photograph of this plant is shown in fig. 3. The results of the above-described experiments appear below in tabular form.

From these experiments with picric acid, alcohol, and $CuSO_4$, we see that it is possible to kill a portion of the stem without completely disorganizing the killed stretch and without interfering with its conducting capacity.

I have further attempted to determine quantitatively the amounts of water that may be evaporated from perfectly dead

TABLE X

SHOWING THE EFFECT OF POISONING 4 TO 15 CM. OF THE STEM WITH XYLOL, PICRIC ACID, ALCOHOL, OR $CuSO_4$

No. of exp.	Height of stem	Distance treated	Time of treatment	Method of treatment	Date	Results and remarks
1—P (1910)...	43 cm.	15 cm.	30 min.	Xylol (and eosin)	April 14	Plant and well-developed branch; involucre remained fresh and turgid for 10 days and branch for 5 days longer; whole plant behaved as though it had had a portion of stem killed by steam; part in tube collapsed and portion killed; leaves yellowed and dried in 15 days.
4—P (1910)...	40 cm.	14 cm.	72 hrs.	Picric acid	April 17	Leaves remained fresh for 7 days; on 8th day leaf tips began to droop, but no signs of withering or fading; plant cut off for examination, acid apparently penetrated stem and carried short distance above place of treatment, but not in leaves, as was shown by color.
5—P (1910)...	35 cm.	4 cm.	48 hrs.	Picric acid	May 3	Plant had healthy crown and one large branch from the crown, 10 cm. long and 2 cm. high; crown leaves drooping in 3 days but turgid and green, showing no fading; May 27, no visible withering or fading; plant cut off and examined; no plugging of the vessels or collapse of parenchyma cells.
6—P (1910)...	40 cm.	10 cm.	48 hrs.	Picric acid	May 3	Plant had good involucre and 3 branches, one 10 cm., one 11 cm., and one 12 cm. long; in 3 days crown leaves slightly drooping, but perfectly turgid; plant still in good condition June 16; removed for examination (see text description).
2—P (1910)...	35 cm.	9 cm.	48 hrs.	95 per cent alcohol	April 12	Plant had 4 branches on crown; crown leaves and 2 branches partially withered in 7 days; in 10 days 6 of crown leaves entirely faded; one branch dry in 10 days; one branch kept one leaf, and one branch kept 2 leaves turgid for 6 weeks; in 10 days after treatment 7 new branches started and were still growing June 18, or 76 days after treatment; stem in tube brown and apparently dead, but not collapsed; cut off for examination; stem fell to pieces where killed.
11—P (1910)...	23 cm.	10 cm.	36 hrs.	Saturated aqueous solution of $CuSO_4$	May 7	Plant had good crown with 2 branches, each 10 cm. long, and 2 others each 8 cm. long; May 15, plant in perfect condition except leaf tips very slightly yellowing; all branches normal; May 20, plant in good condition except one branch; July 9, remaining branches in good condition and growing; July 12, one of remaining branches withered.

plants. It was at once found that the kind of poison used to kill the plant very greatly influences the amount of water evaporated, and that in many cases the amount of water evaporated greatly exceeds the amount transpired by a living plant of the same size and under the same conditions. Cut stems were placed in chromic acid, picric acid, and $HgCl_2$ for 24 hours, until it was certain from their appearance that the poison had been carried to the leaves and that the whole plant was killed; they were then transferred to distilled water. All of the plants in table XI had 17 leaves, each of the same size, age, and area. The area of each plant in table XII was about three times that of each of the plants in table XI, 21 large leaves being on each. Plants of approximately the same leaf areas and of the same stage of development were chosen for controls. The amount of water evaporated from each plant was determined by weighing. Tables XI and XII give the results of my experiments on this point.

TABLES XI AND XII

SHOWING VARIATIONS IN THE DAILY AMOUNTS OF EVAPORATION FROM PLANTS COMPLETELY KILLED BY CHROMIC ACID, PICRIC ACID, OR $HgCl_2$, AS COMPARED WITH A CONTROL PLANT IN WATER

TABLE XI

No. of plant	Fluid	Loss per day in grams										Average loss per day	Percentage of water in plant on 10th day
		1	2	3	4	5	6	7	8	9	10		
I ..	H_2O	0.1	0.9	0.6	0.5	0.5	0.5	0.1	0.4	0.3	0.5	0.44	81
II ..	Chromic	0.1	0.9	0.8	0.8	0.9	0.6	0.5	0.7	0.9	0.5	0.67	36
III ..	$HgCl_2$	1.2	1.1	1.7	1.6	1.5	1.1	0.8	0.9	1.1	1.3	1.23	28
VI ..	Picric	0.7	0.5	0.4	0.4	0.5	0.6	0.4	0.4	0.2	0.4	0.45	11

TABLE XII

No. of plant	Fluid	Loss per day in grams										Average loss per day
		1	2	3	4	5	6	7	8	9	10	
I.....	H_2O	7.4	5.1	3.0	0.9	0.6	0.6	*	*	*	0.5	1.8
II.....	Chromic	2.6	2.9	2.7	2.4	2.5	2.5	*	*	*	3.4	1.9
III.....	$HgCl_2$	7.0	7.6	7.0	6.6	6.5	6.5	*	*	*	10.4	5.2
IV.....	Picric	2.5	1.1	2.7	0.9	2.6	1.8	*	*	*	4.4	1.6

*Impossible to take readings on these days, but does not affect average.

The stems were cut off under water and allowed to remain there for 6 hours, after which they were quickly transferred to the poisons; in each case the base of the stem was immersed for a distance of 5 cm. They were then transferred to bottles containing distilled water, which was covered with a layer of olive oil to prevent surface evaporation. Weighings were made daily at noon for 10 days.

It will be seen from these tables that more water is evaporated from a perfectly dead stem of *Cyperus*, killed with chromic acid, picric acid, or $HgCl_2$, than from a cut plant set in water and kept under the same conditions. These substances in some way apparently alter the constitution of the plant so as to allow it to give off more water. As shown by these tables, a plant cut under water and kept standing in water continually falls off in the amount of water transpired from day to day, and very seldom increases the quantity given off on a certain day over that transpired the previous day. In the case of poisoned plants the reverse is true. Noting the plants numbered II in each table, an increase over the first day is shown on each of the two succeeding days. The amount of water evaporated on the 8th and 9th days exceeds that of the 7th. Certain other days show increases over the preceding days. The plant killed with $HgCl_2$ gives off a quantity of water far in excess of that given off by plants killed by the other two poisons. In table XI plant no. III gives off nearly three times as much water on the average per day as does no. I. Another noticeable fact is that although these poisoned plants evaporate comparatively large quantities of water, the percentage of water contained in the plant is very much below that in plants which have not been killed. This is shown in the last column of table XI; the plant in water contains 81 per cent of water, while the one killed with $HgCl_2$, which gave off the largest quantities of water, contained only 28 per cent of its dry weight of water.

General discussion

As shown by BOEHM (**3**), STRASBURGER (**30**), URSPRUNG (**32–37**), DIXON (**9–12**), ROSHARDT (**24**), and my own experiments, the leaves above a steamed or otherwise heated portion of the stem

being kept in connection with the roots remain for a considerable period turgescent, but sooner or later wither and die. This is very distinctly shown in table IV, from which data it was concluded that the leaves above a steamed portion never remained longer than 18 days without withering. This was shown to be true when only 5 cm. of the stem were steamed, while the leaves remain turgid for 3 days only when 30 cm. were killed. It has been shown that in *Cyperus* sufficient water to maintain the turgidity of the leaves for 3–18 days will rise through a stem 15–60 cm. high with a section 5–30 cm. long, which has been killed by steam. It has also been shown that sufficient water to keep the leaves turgescent for 3 months can ascend through a stem 23 cm. high when 10 cm. are killed with $CuSO_4$. It follows from my observations that the leaves wither above a longer heated stretch faster than above a shorter one, as has been observed by JANSE, URSPRUNG, DIXON, and ROSHARDT. It is further evident that when short portions of the stems are steamed (5–10 cm.), the leaves above do not wither quite as quickly as those on stems cut from the same plant and placed in water under the same conditions of light, temperature, and air moisture. Leaves cut and not placed in water always lose their turgidity and dry long before those on steamed stems, regardless of the length of the killed portion. Such cut branches lose their turgidity in 1–2 hours, and become completely dry in 24–48 hours. The fact that the leaves on heated stems remain longer turgid than those cut and left in air shows that a certain amount of water passes through the killed portion, as is also admitted by URSPRUNG. This is also clearly shown in a quantitative way, by comparing the transpiration after killing a certain portion of the stem with steam with that before the steam was applied. Such a comparison is brought out in tables V and VI. The amount of water passing through such a steamed stem rapidly diminishes, falling off almost immediately from 80 per cent to 50 per cent of the dry weight of the leaves, until the leaves become air dry, when they still contain about 11 per cent of the dry weight of water.

URSPRUNG'S conclusion from such experiments, that living cells are necessary for sap-flow, is certainly not obviously necessary. The long period during which the leaves remain turgid in his own

experiments certainly suggests that the death of the stem cells does not operate in any direct fashion to cut off the water supply. Such a period as 18 days gives opportunity for the development of all sorts of secondary causes, to which the final death of the leaves may well be due. That the amount of water carried is at once reduced may just as well be due to gross mechanical changes secondarily produced in the tissues as to the death of the cells.

DIXON calls attention to the fact that the investigators who first observed the withering of the leaves above a heated portion of the stem, such as WEBER (**40**) and JANSE (**16**), did not attribute the phenomenon simply to the lack of activity of the dead cells of the stem, but to a possible blocking of the vessels, which thus diminishes the water supply. It is certain from the methods which I have used to protect the steamed portions of the stems, that the diminished water supply which reaches the leaves after treatment cannot be due to a lateral evaporation from the heated stretch. The carefully sealed incasing glass tubes preclude such a possibility. URSPRUNG has also concluded from his experiments that the wilting of the leaves above a killed portion of the stem is not due to a lateral evaporation from the heated portion.

In the experiments whose results are shown in tables VIII and IX, there was an increased amount of water transpired on certain days after a section of the stem was killed with heat. It appears, therefore, that URSPRUNG'S statement that the fading of the leaves above a killed portion is a sure index that insufficient water supply reaches them is not proven, and that the wilting may well be due to deleterious substances being introduced into them from the dead cells. Although the plant described in table IX had an increased amount of water ascending to the leaves, they finally withered while still giving off large quantities of water. I am forced to agree with DIXON (**10–12**) that URSPRUNG'S interpretation of these phenomena is an arbitrary one. As noted above, DIXON ascribes the earlier wilting of the leaves on stems, a section of which has been killed with heat, to a possible clogging of the vessels, which hinders water passage, or to a breaking of the water columns due to the heat used, which may thus interrupt the continuity of the flow. He holds, however, that the withering of the leaves is due chiefly

to the action of poisonous or plasmolyzing substances in them, which interferes with the osmotic action of the cells and thus inhibits their lifting power. URSPRUNG regards this suggestion as an empty conjecture.

My results agree well with those of ROSHARDT, except that in the plants treated with steam I have never observed an increase in the amount of water given off during any one period over that in the preceding, such as is shown by ROSHARDT'S table. It appears to me that if the living cells of the stem are necessary to the ascent of water, as ROSHARDT contends, there could not possibly be an increased amount of water transpired during any one interval over that of the preceding. The general gradual falling off in the amount of water transpired after killing a portion of the stem, which ROSHARDT records, agrees with my observations on *Cyperus* under similar circumstances, except that the diminution in the amount of water lost, after steaming a section of the stem, is continuous. I feel that the cause is the same in both cases, namely, a stoppage of the vessels, which I have actually shown to occur in *Cyperus*, and a probable injury to the leaves due to deleterious substances being introduced into them. It seems to me that ROSHARDT has placed exactly the opposite interpretation on the results of his quantitative experiments to that which they really show.

Microscopical examinations of the stems of *Cyperus* above a steamed stretch show that some of the vessels are plugged for a considerable distance with a brown, gumlike gelatinous mass, which reacts to alkanin like resin. This substance is insoluble in water and often stops the vessels throughout the length of the stem above the killed portion, even plugging some of the tracheae of the leaves. There can be no doubt that this stoppage of the vessels in *Cyperus* accounts in a large measure for the immediate and constantly diminished water supply which reaches the leaves above a steamed portion of the stem. The discoloration of the contents of the sieve tubes is conspicuous, and suggests that the steaming causes considerable disorganization, which may be the source of the resinous substances in the vessels. Although URSPRUNG observed stoppage in several plants, he thinks that the

diminished water supply cannot be due to this, since the leaves do not wither at once, but remain for some time turgid. I have described the progressive effect which steaming the stems of *Cyperus* has on the withering of the leaves, but I attribute this progressive injury, which finally results in the death of the leaves, to the introduction of injurious substances from the dead cells. The presence of these poisonous or plasmolyzing substances seems to account for the final withering or drying of the leaves. The leaves first die and then dry, as DIXON puts it. The leaves do not die from lack of water supply, as URSPRUNG would have it, but because they are killed.

Not only are the cavities of the vessels plugged with a brownish mass, but the walls of the conducting tubes are tinged with yellow. It seems quite probable that the presence of some substance in the walls of the tubes may lessen their power of conduction also. DIXON (**10–12**), as noted, has observed the presence of a substance which stains the walls of the vessels and thinks that "it would be hard to believe that the deposit of this colored substance in the walls and lumina of the tubes could be without effect on their efficiency in transmitting water." I have already described a similar brownish appearance of the walls of the vessels as well as the plugging of the lumina for some distance above the cut end of a stem of *Cyperus* standing in a sterilized decoction of the same plant. SCHWENDENER'S (**28**) researches, tending to show that heating the stem does not change the physical character of the cell walls of the tubes, and that the micellar structure and imbibitional power is not affected by such treatment, probably do not take sufficient account of the action of poisonous substances caused by heating the stem on the structure of the walls of the vessels and the efficiency of their conductivity. From my experiments it is clear that changes in conductivity and in the amount of evaporation are brought about by killing the plant with such substances as chromic acid, picric acid, and $HgCl_2$, often accelerating evaporation to a very marked degree.

I have repeatedly shown that when stems are set in a decoction of the same plant, the leaves wither much earlier than those set in water. Additional evidence that injurious substances are engendered by heating the plant was obtained by growing *Cyperus*

plants in nutrient solutions containing a decoction. As noted above, the plants grown in such solutions began to droop in 3–5 days, showing discoloration and fading along the veins in 7–8 days, while control plants grown in nutrient solutions without the decoction remain perfectly normal. DIXON'S experiment, in which leaves normally supplied with water together with water from a heated side branch were shown to soon wither, seems to me quite decisive proof that deleterious substances enter the leaves from the killed portion. URSPRUNG (**35–37**), however, was unable to produce the same effect on the leaves of *Impatiens* by using DIXON'S method.

Microscopical examination of the leaves from a stem killed by heat show in certain regions a discoloration of the mesophyll cells, the protoplasts being contracted and the chloroplasts being discolored. The leaves of plants grown in decoctions also show similar conditions. DIXON (**10–12**) also found disorganization and discoloration of the mesophyll cells of the leaves above a killed portion, and, as noted, even on a separate branch if some of its water supply passes through a heated region. In these cases it certainly appears that the leaves are drying not so much for lack of water as from injury and death of the cells. The observation of SCHROEDER (**27**) that most leaves can lose 50 per cent of their fresh weight without injury is further proof that leaves above a heated portion of a stem do not wither on account of the diminished water supply. In the microscopical examinations which I have made of leaves from steamed stems, I have found many of the conditions described by SCHROEDER in his studies on the symptoms of death as a result of wilting, namely, the contraction of the protoplasts of the mesophyll, and the change in color and rounding up of the chloroplasts. The fact that the plant killed with steam constantly decreases in the amount of water given off is also in harmony with SCHROEDER'S observations. The first 50 per cent of the leaf's fresh weight is very rapidly lost when dying, as he shows, after which the amount lost decreases uniformly. My experiments, in which the amount of transpiration was determined after killing a portion of the stem with steam, show that there is in the first 2 or 3 days a very rapid and immediate decrease in the water loss, and that then the rate of transpiration

decreases more uniformly. These facts, taken together with the appearance of the mesophyll, seem to show beyond question that the leaves on a steamed stem are dying, and that death is not entirely due to a lack of water.

Applying heat by means of wax heated to 110° C. does not cause so much discoloration of the contents of the sieve tubes, or so much stoppage of the lumina of the vessels, and the mesophyll of the leaves does not show so much immediate plasmolysis. The leaves above stems so treated remain turgid three times as long as those on steamed stems. Plants do not show such an immediate decrease in the transpiration as is the case when the stems are killed with steam. The effect upon the transpiration rate and upon the injury to the leaves in using hot wax is not a progressive one, as it is when steam is used. Altogether the method of using hot wax to kill a section of the stem seems more satisfactory than steaming, with reference to the question of the relation of the living cells to sap-flow. And it is still plainer in stems killed in this way that the death of the stem cells does not *per se* directly affect the sap-flow.

My experiments with poisons, in which 5–10 cm. of the stem were treated for 36–72 hours, show that it is possible to kill a portion of the stem without completely disorganizing the killed stretch and without reducing its conducting capacity. Not only does a sufficient quantity of water pass through the poisoned portions to supply the transpiration needs for a comparatively long period of time (3 months in the case of $CuSO_4$), but also to allow the development and growth of new parts. As has been shown, the mesophyll cells remain perfectly normal; no discoloration of the chloroplasts and no contraction of the protoplasts follow this method of treatment, if care is taken that the poison does not reach the leaves. In the poisoned portion of the stem the cells are apparently "fixed" when alcohol or picric acid is used, there being no plasmolysis. When $CuSO_4$ is used, the parenchyma of this region has its protoplasts contracted, but the vessels remain normally open and apparently unaltered.

From my experiments, in which poisons were used to kill the whole plant, it was at once evident that the kind of poison greatly influenced the subsequent rate of evaporation of water from the

whole plant, and that in many cases the new rate far exceeds the normal transpiration of a plant of the same age and superficial area under the same conditions. In these cases it is plain that the tissues are ruptured so as to expose additional cell surfaces to the atmosphere.

DIXON and JOLY (**13**) consider that capillarity or imbibition of the mesophyll cell walls sets up a suction, aiding the osmotic suction in extracting water from the adjacent vessels. In the case of the poisoned leaves of *Cyperus* there can be no osmotic action of the cells. The imbibitional action of the cell walls, as conceived by DIXON, may however still keep the walls wetted, and the suction from the evaporation may be transmitted to the cohering water columns of the vessels. DIXON (**11**) points out that the point of support for the tensile strength in the case of transpiring dead organs is always the walls. He thinks that the presence of soluble substances on the outer surface of the walls would function also in maintaining the suction. Perhaps the condensation of the metallic salts of these poisons on the outer surfaces of the cell walls act in this manner.

BOEHM (**4**) formulated a theory of sap-flow based entirely upon capillarity, maintaining that the capillary attraction between the walls of the conducting tracts and the water is greater than obtains in a glass tube of the same diameter. The experiments of STRASBURGER (**30**), however, show that in the vessels of *Aristolochia* the capillary ascent of water is much slower than in glass tubes of the same diameter. It seems quite possible that $HgCl_2$, and some of the other poisons used by me, which cause an increased amount of evaporation, as is shown in tables X and XI, may in some way alter this capillary relation so that the water may flow faster in such poisoned tubes. Cells without turgor, that is perfectly dead cells of the leaves, are able to raise water to a considerable height. STRASBURGER showed that rather tall trees with poisoned leaves are able to raise water 22 m. ASKENASY (**1**) found that cut branches of *Taxus* and *Viburnum*, whose lengths he does not mention, which had lain for a long time in boiling water or in alcohol and were completely dead, could suck up water and eosin. BOEHM (**4, 5**) also showed that water could rise in a dead

branch when the leaves were dead. A cooked and dried oak twig about 25 cm. long was then set in a tube with a manometer, with water above and mercury below. The twig was able to raise mercury 70.3 cm. high. In another somewhat modified experiment with a *Thuja* twig the mercury rose to 86.4 cm.

ASKENASY believes that the suction power exhibited by dead leaves is explainable by the fact that the water evaporates from the outer surface of the cell walls of the mesophyll into the intercellular spaces, and is supplied from within through the protoplasmic lining. He thinks that the imbibitional force of the wall is greater than the osmotic power of the cell sap. He further adds:

> Da diese Imbibitionskraft durch den Tod der Zelle im Allgemeinen nicht beeinträchtigt wird, so ist es kein Wunder, dass auch tote Zellen, wenn sonst die Verhältnisse günstig liegen, im Stande sind, das an ihnen verdunstende Wasser ebenso hoch zu heben wie lebendige.

There can be no doubt that cell walls possess a great attraction for water. As DIXON (**10–12**) points out, the submicroscopical spaces occupied by the imbibed water in the cell walls of the mesophyll are intensely minute capillaries. It seems quite possible that these poisons may in some way alter the character of these passages, and so affect the amount of water imbibed by the walls and also the amount of water evaporated by the surfaces of these cells. It is certain that in the case of plants poisoned throughout, the elevation of the water in the stems, and its evaporation from the leaves in larger quantities than normally occurs in living plants, depend purely upon physical processes.

Summary

1. Stems of *Cyperus* cut and placed in water wither sooner than when a certain portion, not to exceed 20 cm., has been killed by steam.

2. When 20 cm. of the stem are killed by steam, the leaves wither in about 8 days, that is, in about the same time as the control plants.

3. The longer the portion of the stem killed with steam, the sooner the leaves above wither and dry. When 25 to 30 cm. of the stem are killed with steam, the leaves wither in 3–5 days.

4. No matter how long the section killed may be, the leaves on steamed stems never wither quite so quickly as those cut and not placed in water, but under the same conditions of light, temperature, and air moisture.

5. In *Cyperus* sufficient water to maintain the leaves turgid for 3–18 days will rise through a stem 15–60 cm. high, with a section 5–30 cm. long which has been killed with steam.

6. A certain amount of water is raised through the steamed portion, but it gradually diminishes in quantity from day to day, until the leaves become air dry (about 11 per cent of their dry weight of moisture).

7. The diminished water supply is partly due to a partial blocking of the vessels with a gumlike or resinous substance, which probably owes its origin to the disorganization of the contents of the sieve tubes caused by heating the stems.

8. The withering of the leaves above a steamed portion of the stem is probably caused more by the action of deleterious substances introduced into them from the dead cells than from lack of water. These poisonous substances are probably disorganization products caused by heating with steam.

9. The leaves of rooted plants, grown in nutrient solutions containing sterilized decoctions of the same plant, droop in 3–5 days, discolor and dry in 7–8 days.

10. The withering leaves above a portion of the stem killed with steam show all the symptoms of dying, namely, rapid loss of water after treatment, then a more uniform loss, rounding up and discoloration of the chloroplasts, and contraction of the mesophyll protoplasts. The leaves are apparently drying, not so much from lack of water as on account of the death of the cells from other causes.

11. Judging from the behavior and disorganization of the leaves on a stem, a section of which has been killed with steam, it is evident that this method of killing the cells is not a satisfactory one in order to settle the question as to the relation of the living cells to sap-flow.

12. Killing a portion of the stem by applying wax heated to 110° C. causes less apparent disorganization of the cells, less injury

to the leaves above, and does not cause a marked immediate decrease in the transpiration.

13. Experiments in which 5–10 cm. of the stem are killed by treatment with picric acid, 95 per cent alcohol, or $CuSO_4$, for 36–48 hours show that sufficient quantities of water may ascend through the poisoned portions to supply the transpiration need for a comparatively long period (90 days), and to allow the development of new branches.

14. Certain poisons (picric acid, chromic acid, and $HgCl_2$) may greatly accelerate the amount of water evaporated in poisoned plants. Not all poisons act alike in this respect; $HgCl_2$ causes the greatest amount of increase in water loss.

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